

294-102 PCT/US

09/869409

INTERNATIONAL APPLICATION NO.
PCT/NL99/00804INTERNATIONAL FILING DATE
24 December 1999PRIORITY DATE CLAIMED
06 January 1999

TITLE OF INVENTION

Accessing Leaf and/or Stem Parts of Plants

APPLICANT(S) FOR DO/EO/US

Anne Coenraad HULST, Jan Josef Maria Hubert KETELAARS, and Johan Pieter Marinus SANDERS

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. is attached hereto (required only if not communicated by the International Bureau).
 - b. has been communicated by the International Bureau.
 - c. is not required, as the application was filed in the United States Receiving Office (RO/US).
6. An English language translation of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. is attached hereto.
 - b. has been previously submitted under 35 U.S.C. 154(d)(4).
7. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. are attached hereto (required only if not communicated by the International Bureau).
 - b. have been communicated by the International Bureau.
 - c. have not been made; however, the time limit for making such amendments has NOT expired.
 - d. have not been made and will not be made.
8. An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. A **FIRST** preliminary amendment.
16. A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. A substitute specification.
18. A change of power of attorney and/or address letter.
19. A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. Certificate of Mailing by Express Mail
23. Other items or information:

EXPRESS MAIL CERTIFICATE

Date 06/25/01 Label No. EL470332425US

I hereby certify that on the date indicated above, I deposited this paper or fee with the U.S. Postal Service & that it was addressed for delivery to the Assistant Commissioner for Patents, Washington, D.C. 20231 by "EXPRESS MAIL Post Office to Addressee" Service.

Cassandra Lems
Name (Print) Cassandra Lems
Signature

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 09/869409	INTERNATIONAL APPLICATION NO. PCT/NL99/00804	ATTORNEY'S DOCKET NUMBER 294-102 PCT/US
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24. The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

<input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	\$1000.00
<input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	\$860.00
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	\$710.00
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	\$690.00
<input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	\$100.00

CALCULATIONS PTO USE ONLY

ENTER APPROPRIATE BASIC FEE AMOUNT =

\$860.00

Surcharge of **\$130.00** for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492 (e)).

20 30

\$0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	20 - 20 =	0	x \$18.00	\$0.00
Independent claims	1 - 3 =	0	x \$80.00	\$0.00
Multiple Dependent Claims (check if applicable).			<input checked="" type="checkbox"/>	\$270.00

TOTAL OF ABOVE CALCULATIONS =

\$1,130.00

Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.

\$0.00

SUBTOTAL =

\$1,130.00

Processing fee of **\$130.00** for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492 (f)).

20 30

\$0.00

TOTAL NATIONAL FEE =

\$1,130.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).

\$0.00

TOTAL FEES ENCLOSED =

\$1,130.00

Amount to be: refunded	\$
charged	\$

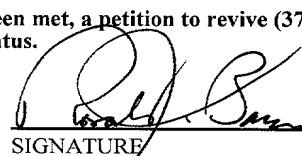
- A check in the amount of **\$1,130.00** to cover the above fees is enclosed.
- Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.
- The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **08-2461** A duplicate copy of this sheet is enclosed.
- Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

Ronald J. Baron

NAME

29,281

REGISTRATION NUMBER

25 June 2001

DATE

13 Rec'd PCT/PTO 06 AUG 2001
09/869 409
PATENT



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Hulst, et al.

Examiner: Unassigned

Serial No: 09/869,409

Group Art Unit: Unassigned

Filing Date: June 25, 2001

Docket: 294-102 PCT/US

For: ACCESSING LEAF AND/OR
STEM PARTS OF PLANTS

Dated: August 1, 2001

Assistant Commissioner for Patents
Washington, DC 20231

PRELIMINARY AMENDMENT

Sir:

Applicants respectfully submit the following Preliminary Amendment for entry in the above-identified application prior to examination.

IN THE SPECIFICATION:

On page 1, before line 1, please insert the following before the first paragraph:

BACKGROUND OF THE INVENTION

On page 6, line 16, please inset the following:

SUMMARY OF THE INVENTION

On page 8, before line 1, please insert the following before the first paragraph:

DETAILED DESCRIPTION OF THE INVENTION

IN THE CLAIMS:

Please cancel Claim 1 and insert therefor the following new Claim 19:

19. (New) A method for separating components from vegetable material having at least leaf and/or stem parts comprising:
 - a) at least partially fiberizing said vegetable material and
 - b) subsequently separating said at least partially fiberized material into
 - i) a fiber fraction comprising relatively firm tissues, such as epidermis, sclerenchyma, and vascular bundles, and
 - ii) a juice stream comprising soft tissues, such as parenchyma and cytosol.

Please amend Claims 2, 3, 5 and 6 to read as follows:

2. (Amended) A method according to claim 19, wherein the juice stream comprises chloroplasts.
3. (Amended) A method according to claim 19, wherein the material is mechanically fiberized.
5. (Amended) A method according to claim 19, wherein the fiber fraction is separated from the juice stream comprising one of screening, centrifugation, processing by hydro(cyclone), centriscreening, decanting, sedimentation, or combinations thereof.
6. (Amended) A method according to claim 19, wherein the vegetable material originates from a cultivated crop.

Please cancel Claim 8-18 without prejudice.

Please add new Claims 20-41, as follows:

20. (New) A fiber fraction obtained by a method for separating components from vegetable material having at least leaf and/or stem parts comprising:

- a) at least partially fiberizing said vegetable material and
- b) subsequently separating said at least partially fiberized material into
 - i) a fiber fraction comprising relatively firm tissues, such as epidermis, sclerenchyma, and vascular bundles, and
 - ii) a juice stream comprising soft tissues, such as parenchyma and cytosol.

21. (New) A biodegradable product comprising a fiber fraction obtained by a method for separating components from vegetable material having at least leaf and/or stem parts comprising:

- a) at least partially fiberizing said vegetable material and
- b) subsequently separating said at least partially fiberized material into
 - i) a fiber fraction comprising relatively firm tissues, such as epidermis, sclerenchyma, and vascular bundles, and
 - ii) a juice stream comprising soft tissues, such as parenchyma and cytosol.

22. (New) A biodegradable product according to claim 21, wherein said product is paper.

23. (New) A biodegradable product according to claim 21, wherein said product is cardboard.

24. (New) A biodegradable product according to claim 21, wherein said product is fiberboard.

25. (New) A biodegradable product according to claim 21, wherein said product is used in the preparation of a moisture absorbing material.

26. (New) A biodegradable product according to claim 21, wherein said product is used in the preparation of growth media.

27. (New) A biodegradable product according to claim 21, wherein said product is a soil improver.

28. (New) A biodegradable product according to claim 21, wherein said product is fuel to produce energy.

29. (New) A juice stream obtained by a method for separating components from vegetable material having at least leaf and/or stem parts comprising:

- a) at least partially fiberizing said vegetable material and
- b) subsequently separating said at least partially fiberized material into
 - i) a fiber fraction comprising relatively firm tissues, such as epidermis, sclerenchyma, and vascular bundles, and
 - ii) a juice stream comprising soft tissues, such as parenchyma and cytosol.

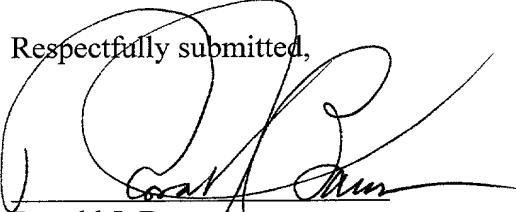
30. (New) A juice stream according to claim 29, which contains more than 55% of the crude protein of the vegetable material.
31. (New) A juice stream according to claim 30, which contains preferably more than 75% of the crude protein of the vegetable material.
32. (New) A juice stream according to claim 31, which contains preferably more than 90% of the crude protein of the vegetable material.
33. (New) A protein enriched product comprising a juice stream obtained by a method for separating components from vegetable material having at least leaf and/or stem parts comprising:
 - a) at least partially fiberizing said vegetable material and
 - b) subsequently separating said at least partially fiberized material into
 - i) a fiber fraction comprising relatively firm tissues, such as epidermis, sclerenchyma, and vascular bundles, and
 - ii) a juice stream comprising soft tissues, such as parenchyma and cytosol.
34. (New) A protein enriched product according to claim 33, wherein said product is food.

35. (New) A protein enriched product according to claim 34, wherein said food is for human nourishment.
36. (New) A protein enriched product according to claim 34, wherein said food is for use in livestock feeding.
37. (New) A protein enriched product according to claim 33, wherein said product is a substrate for fermentation.
38. (New) A protein enriched product according to claim 33, which results from recovering or purifying a substance contained in said juice stream.
39. (New) An apparatus for separating components from vegetable material having at least leaf and/or stem parts comprising:
 - a) a fiberizer which dissociates relatively firm tissues from the relatively soft tissues of said vegetable material, and
 - b) a separator which separates the fiber fraction from the juice stream.
40. (New) An apparatus according to claim 39, wherein said apparatus comprises a refiner.
41. (New) An apparatus according to claim 39, wherein said apparatus comprises a blender.

REMARKS

Applicants have undertaken to cancel Claims 1 and 8-18, amend Claims 2, 3, 5, and 6, and add new Claims 19-41 in the above-identified application in order to remove improper multiple dependencies and conform to U.S. practice. No new matter has been added. In addition, headings have been added to the specification. Accordingly, entry hereof and examination on the merits are respectfully requested.

Respectfully submitted,


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Application No. 09/869409
Filing Date: June 25, 2001
Docket No.: 294-102 PCT/US
Page 8

VERSION OF AMENDMENT WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

On page 1, before line 1, please insert the following before the first paragraph:

BACKGROUND OF THE INVENTION

On page 6, line 16, please inset the following:

SUMMARY OF THE INVENTION

On page 8, before line 1, please insert the following before the first paragraph:

DETAILED DESCRIPTION OF THE INVENTION

IN THE CLAIMS:

Please amend Claims 2, 3, 5 and 6 to read as follows:

2. (Amended) A method according to claim 19 [1], wherein the juice stream comprises chloroplasts.

3. (Amended) A method according to claim 19 [1 or 2], wherein the material is mechanically fiberized.

5. (Amended) A method according to claim 19 [any one of claims 1-4], wherein the fiber fraction is separated from the juice stream [through] comprising one of screening, centrifugation, processing by hydro(cyclone), centriscreening, decanting, sedimentation, or combinations thereof.
6. (Amended) A method according to claim 19 [any one of claims 1-5], wherein the vegetable material originates from a cultivated crop.

Title: Accessing leaf and/or stem parts of plants.

The invention relates to the separation and recovery of components from vegetable raw materials.

Plants, like most organisms, are made up of cells. A plant cell consists of a lipid membrane with a generally aqueous content, the cytosol, which contains the various cell organelles (likewise surrounded by lipid membranes), such as nucleus, mitochondria, endoplasmic reticulum and chloroplasts, and the cytoskeleton, made up of microfilaments and microtubules, which gives the cell an inner structure. Also present in the plant cell are vacuoles which play an important role in keeping the plant cell under tension; the vacuoles maintain the turgor of the cell.

The constituent components of a plant cell can be roughly distinguished into water, which accounts for the greater part by far of a living cell, components such as salts, (precursors of) lipids, carbohydrates, amino acids and nucleotides, macromolecules such as starches, proteins and nucleic acid and a multiplicity of other molecules, including vitamins and pigments such as chlorophyll, carotene and xanthophyll.

A plant cell is generally surrounded by a cell wall which provides firmness and structure to the plant tissue. The cell wall is mainly built up from (hemi)cellulose and other carbohydrate polymers, which have aggregated to fibers. Woody plants further contain an ample amount of lignin, a polymer made up of phenols and other aromatic monomers.

Plant tissue is made up of plant cells, all of which, when living, basically satisfy the above description. An important distinction can be made between relatively firm tissues which comprise virtually no chloroplast or other plastid containing cells, and the relatively soft tissues which generally do. Tissues which generally comprise no chloroplast containing cells are, for instance, the epidermis or skin tissue of a plant, the collenchyma and sclerenchyma or stroma of a plant and the vascular

fiber bundles or the vascular tissue, comprising the important transport vessels (wood vessels and sieve tubes) in the plant. When a part of a plant is strongly lignified, in general, over time, the majority of the cells in the lignified part die off and only residues of the cell content are left. In

5 particular the cytosol and the organelles present therein are lost, but the vascular fiber bundles, skin and stromas generally give the plant form and structure and generally remain present when the plant is dead.

Characteristically, these relatively firm tissues (in particular vascular bundles, sclerenchyma and epidermis) comprise no to virtually no 10 chloroplast containing cells, while an important part (at least in the aerial leaf and stem parts of the plant) of the relatively soft tissues, also called chlorenchyma, is made up chiefly of only chloroplast-containing parenchymal cells; indeed, this is where photosynthesis occurs.

It has long been known to recover various components from 15 vegetable raw materials for further use in, for instance, food for human or feed for animal consumption through mechanical methods. Often, plants are merely comminuted or chopped to make them suitable for consumption, an example being the chopping of maize for feed.

However, in particular the components present in the plant cell 20 cytosol are outstandingly suitable for human food or animal feed, since these can be building materials for corresponding components which are found in animal cells.

Mechanical processing is applied, for instance, to feed crops, such as grass, lucerne and other fresh and green harvested plants which, often as 25 virtually whole plant, in particular the leaf and/or stem parts and in most cases not including the roots, are used for recovering, for instance, (animal) feed components. Such vegetable raw materials are generally recovered through pressing of (preferably chopped or otherwise comminuted) leaf and/or stem material, whereby a part of the vegetable 30 material is obtained as press juice, while the residual and pressed material is known as press cake.

The pressure forces exerted by pressing generally result in the opening up (snapping or bursting) of plant cells in the material, so that the aqueous but food component-rich cytosol, possibly with residues of the organelles and the lipid membrane surrounding the cell, is liberated from the cell as press juice. Press juice is generally treated further, for instance through screening, whereafter, for instance, the protein in the juice is recovered by means of coagulation through, for instance, acid and/or heat treatment. Press juice may also be further processed through (ultra or membrane) filtration, drying, fermentation or other methods known to the skilled person. Protein-rich or otherwise high-grade nutrients for human and animal consumption, but also pigments such as carotene (provitamin A), can be recovered from cytosol in this way.

The resultant, relatively dry press cake is generally considered to be less rich in food; it contains relatively intact fiber bundles composed of (not directly) digestible cellulose fibers, adherent press juice and residual plant cells which have not been accessed under the influence of the pressing. Especially these residual plant cells with unrecovered cytosol give fodder value to the press cake, which is generally dried and, pelleted or otherwise, is used as relatively low-grade roughage component in fodders, in particular for ruminants.

For mechanically accessing, for instance, lucerne or grasses, traditionally a method is used which is based on the disintegration of the vegetable raw material by means of hammer mills followed by squeezing the disintegrated raw material (here designated as pulp) using screw presses or belt presses. The pulp is thereby separated into a press cake fraction and a press juice fraction. The juice fraction is regarded as the fraction in which the industrially recoverable content substances from the plant material are contained. Hammer mills typically consist of a rotor on which fixed or freely movable elements are disposed which upon rotation of the rotor are brought into contact with the vegetable raw material and disintegrate it through force of impact. The disintegratory effect of hammer mills is relatively large when the vegetable material has a good

turgor, i.e. when the plant cells are under tension. In that case, the force of impact causes the tissue to snap and causes the cell content constituents to be liberated with the tissue fluid. If turgor is low, beating the plant material will cause it to be compressed. The tissue then remains more or 5 less intact and the result is that the cell content becomes available to a much lesser extent. This has major consequences for the recoverability of particularly those cell content constituents that are present in the vegetable biomass only partly in dissolved form and for another part in the form of solid, undissolved matter. This holds true *inter alia* for 10 vegetable proteins, but also for lipids and pigments. Also known (for instance from US 5,464,160) are hammer mills where relatively dry material is separated into two fractions, this while neglecting the so valuable juice stream with protein-rich cytosol. Accordingly, these types of mills are not suitable for processing fresh, relatively wet material and 15 eventually produce a relatively wet fiber fraction.

In the above-described methods of pressing vegetable material which contains at least leaf and/or stem parts, it is generally of importance that the material be processed while still as fresh as possible, shortly after harvesting. Only then are the plant cells sufficiently under 20 tension to be able to burst or snap under pressure so that the cytosol is liberated. When, after harvesting, already some time has elapsed before the plant parts are pressed, they are already dried out to some extent by then, the plant cells present have lost a large part of the necessary turgor and are too slack to be able to snap or burst under pressure. Accordingly, 25 in non-fresh material, the recovery of press juice will proceed with less efficiency. The same holds for material stemming from plants which, even before they were harvested, already lost a large part of the turgor in their plant cells through drying out and/or maturation. In general, such plants are not (completely) green anymore but acquire brown or yellow aspects. 30 Lignified plant parts are altogether ineligible for the above methods, since most cells have died off in them, or in any case they contain only a very

minor cytosol fraction and hence do not contribute to the recovery of high-grade food.

In general, plant material is separated into a press cake fraction and a press juice fraction. Characteristic of this method is the only partial extraction (along with the press juice) of the cell content constituents (vacuole content and cytoplasm with cell organelles present therein, such as chloroplasts and cell nuclei); the cell walls are substantially completely left behind in the press cake together with the remainder of the cell content. Contained in the press cake are all tissues which are also contained in the raw material, and in addition also a part of the cell content. The color of the fresh press cake is predominantly green in that the chloroplasts having therein the chlorophyll (leaf green) present, have only been partly removed with the press juice. The plant material has only partly disintegrated down to tissue level; this means that still recognizable fragments of leaves and stems are present in addition to individual tissues such as isolated vascular bundles.

The press juice consists substantially of the aqueous content of cells: the vacuole content and the cytoplasm having therein cell organelles such as chloroplasts in intact or disintegrated form; cell wall constituents are substantially absent in that they remain behind in the press cake.

Consequently, the recoverability of protein and other partly soluble substances in the traditional method of fractionation is highly susceptible to variations in the nature of the vegetable biomass, in particular the presence of turgor, which is typically reflected in differences in dry matter content.

The traditional method of fractionation has as a consequence that upon squeezing the pulp, only a part of the cell content constituents end up in the juice stream and another part remains behind in the press cake. Accordingly, the press cake still contains, in addition to the greater part of the cell walls, a part of the cell content constituents and, by virtue of that, is used as fodder.

The existing pressing methods for separating high-grade from low-grade components from vegetable material are therefore relatively strongly dependent on the turgor of the cells present in the vegetable material, which limits the application of these methods to their 5 application to relatively fresh and green material. Often, the resultant press cake, also when fresh and/or green material is used, still contains large amounts of unaccessed plant cells with high-grade cytosol in them, while only a low price can be obtained for press cake since it is in fact suitable only as a relatively low-grade component of animal fodder. For 10 recovering high-grade components from leaf and/or stem parts, there is a need for better methods which can access the plant cell with a higher efficiency than the existing methods, can make the cytosol fraction more available for recovery, and affords better marketing possibilities for the fiber-containing residual material. The object of the invention is to provide 15 for this need.

The invention provides a method for separation of components from vegetable material, such as leaf and/or stem parts, characterized in that the material is at the least partly fiberized and subsequently is separated 20 into a fiber fraction and a juice stream, such that the fiber fraction chiefly comprises relatively firm tissues such as epidermis, sclerenchyma and vascular bundles, and the juice stream chiefly contains soft tissues such as parenchyma, and cytosol. In a preferred embodiment, the invention provides a method for separating a juice stream comprising in particular 25 parenchyma with chloroplasts.

The invention provides a new method of fractionation which consists of at least two steps: a first step in which the vegetable material is fiberized through the action of shear forces and a second step in which the fiber fraction is separated from the rest. Fractionation of vegetable 30 biomass means the separation into a number of fractions. By fractionating biomass, new product streams are formed with other application possibilities than the raw material itself. Consequently, these new product

streams jointly often represent more value than the original biomass. The invention provides a new technique which is based on fiberization and subsequent defibration of vegetable biomass.

In a preferred embodiment, the invention provides a method for separation of components from vegetable material, characterized in that the material is at the least partly mechanically fiberized and subsequently is separated into a fiber fraction and a juice stream, with the fiber fraction (see, for instance, Figs. 1 and 2, also for a comparison with a traditional method) principally comprising relatively firm tissues such as epidermis, sclerenchyma and vascular bundles, and the juice stream (see, for instance, Figs. 6 and 7, also for a comparison with a traditional method) principally containing soft tissues such as parenchyma, and cytosol. The mechanical fiberization is effected, for instance, through treatment of the material in a blender. Preferably, certainly when application on an industrial scale is desired, the fiberization is done, according to the invention, with an apparatus such as a (pressure) refiner, with grinding disks, such as employed in the pulp and paper industry, or in an apparatus of equivalent action by which the vegetable material can be fiberized to enable separation into a fiber fraction which principally comprises relatively firm tissues such as epidermis, sclerenchyma and vascular bundles, and the juice stream principally comprising soft tissues such as parenchyma, and cytosol.

The method according to the invention is applicable to all fiber containing vegetable materials, originating both from cultivated plants (crop plants) and from wild plants, as well as to crossing products of cultivated plants among themselves or with wild plants. Examples are: vegetable biomass originating from cultivated grassland, but also from natural grounds and roadsides, feed crops such as forage grasses and corn, lucerne, clover, and other papilionaceous plants, fiber crops such as flax and hemp, and the tops of crops normally grown solely for their seeds, fruits or tubers, such as grains, beets, peas, beans, potatoes, carrots, cassava, sweet potato.

In fiberization, the vascular tissue with the sclerenchyma and the epidermis (jointly the fiber fraction) is mechanically dissociated from the other, substantially parenchymal tissue. This parenchymal tissue is at the same time accessed and the cell content constituents from it thereby

5 become available substantially completely. Fiberization can be done using refiners such as they are in use in the pulp and paper industry for fiberizing wood and wood pulp. Refining, or fiberization, typically occurs with addition of moisture to the plant material. The result is then a slurry of fiberized material from which the fibers can be removed. The fiber

10 fraction (fiber stream) which is thus recovered, is suitable, through its nature and composition, inter alia for the following applications: as raw material for paper and cardboard (solid cardboard, folding cardboard and form cardboard), as raw material for the production of fiberboard materials (softboard, hardboard, chipboard, MDF, HDF and MDF/HDF

15 form parts) and composites, as raw material for moisture absorbing materials, such as diapers, sanitary napkins, and so forth, as raw material for the preparation of growth media (potting compost and substrates), mulches (as protection against erosion, and as weed and disease suppressant), as soil improver or as fuel.

20 In defibration, the liberated fiber is separated, for instance through screening, from the other plant constituents. Through washing and screening, the fiber can be further purified and as many non-fiber constituents as possible can still be recovered with the washing water. The defibered slurry then consists of a mixture of added water, tissue fluid, cell

25 content constituents and finely dispersed cell walls coming from the parenchymal tissue. From the defibered slurry or juice stream, content substances can be recovered in a more or less pure form, such as: proteins, peptides and amino acids, enzymes, pigments, lipids, fatty acids, starches, soluble sugars and (cell wall) carbohydrates for use in livestock feeding,

30 human nourishment, or as substrate for fermentations, or, through concentration, fodder or food products can be made with a high nutritive value as a result of the removal of the non-digestible or poorly digestible

fiber fraction. The defibered slurry can be further fractionated in subsequent steps. One possibility is, for instance, the separation of all solid parts through centrifugation, which may or may not be preceded by a coagulation step through heating, acidification or otherwise. Another 5 possibility is to convert the parenchymal cell walls into soluble sugars using cell wall splitting enzymes (pectinases, cellulases, etc.) and thus adding them to the fraction of dissolved substance in the defibered slurry.

Characteristic of the method as provided by the invention is the split at tissue level into a fiber fraction which contains the relatively firm 10 tissues (vascular bundles, sclerenchyma and epidermis) and a defibered fraction which contains the relatively soft tissues (parenchyma) with their content. Briefly summarized, the difference between the traditional and novel method is the extraction of tissue fluid (traditional) versus tissue fractionation (new method).

15 The invention also provides an apparatus for practicing a method according to the invention. Such an apparatus is characterized by means suitable for the fiberization according to the invention, whereby the relatively firm vascular tissue with, for instance, the sclerenchyma and the epidermis (jointly the fiber fraction) is mechanically dissociated from 20 the other, substantially parenchymal tissue. This parenchymal tissue is at the same time accessed and the cell content constituents therefrom thereby become available substantially completely. 'Fiberization' is herein understood to mean that the plant material is exposed to such forces that the relatively firm tissues are dissociated virtually completely from the 25 relatively soft tissues. As a resultant of the forces which effect this fiberization, the great majority, if not virtually all, of the plant cells will be accessed, so that the cytosol is liberated. This cytosol, as a juice stream generally also including residues of the organelles and the cell surrounding lipid membrane and parenchymal cell walls, can be relatively 30 simply separated from the fiber component through screening or through other separation means known to those skilled in the art.

A first advantage of the invention is that the efficiency of the method is not dependent on the turgor of the plant cells present in the material, so that the plant cells can be accessed with greater efficiency than is conventional in the above-described pressing methods.

5 A second advantage of the invention is that the invention provides two product streams which as such are very pure. A first one, the fiber fraction, contains principally cellulose and hemicellulose, principally consisting of the elements C, H and O (which in itself yields advantages for a clean combustion); a second one contains all valuable and complex
10 content substances to be found in the parenchyma and cytosol, and which can be further separated relatively simply.

The two product streams can be separated from each other by, for instance, screening. Other separation methods than screening are also conceivable, for instance centrifugation, processing by (hydro)cyclone and
15 centriscreening, and decanting or sedimentation, or combinations of these methods. In defibration, the liberated fiber is separated from the other plant constituents through, for instance, screening. By washing and screening, the fiber can be further purified and as many non-fiber constituents as possible can still be recovered with the washing water. The
20 defibered slurry then consists of a mixture of added water, tissue fluid, cell content constituents and finely dispersed cell walls coming from the parenchymal tissue.

A first product stream as contemplated by the invention is a (generally nutritionally high-grade) juice stream consisting of an aqueous
25 solution/suspension of virtually all high-grade components or nutrients from the vegetable material (such as sugars, fructose-oligosaccharides, proteins, lipids, pigments, and the like). Through removal of the (nutritionally low-grade) fiber components, there is formed (on a dry matter basis) this relatively high-grade product stream, from which the
30 various components can be further isolated relatively simply. The defibered product or the juice stream consists substantially of parenchyma, partly as intact cells, partly as disintegrated cell material.

The color of the defibered product is typically green due to the presence of intact or broken chloroplasts, sometimes brown-green through browning during the fractionation. Macroscopically, it is a liquid. Microscopically, principally intact and disintegrated parenchyma cells and cell organelles such as chloroplasts are visible in this liquid.

The second product stream, the fiber fraction as contemplated by the invention, consists of the relatively hard tissues. These are typically the vascular bundles, the sclerenchyma and the epidermis. The cell content is absent from these tissues or is removed virtually completely during fractionation and washing. Consequently, fiber consists predominantly of cell wall components. Chloroplasts are virtually absent in a pure fiber preparation. The color of the washed fiber typically varies from white to yellow or light-brown. Sometimes, a light-green color may arise as a result of impregnation with chlorophyll during recovery.

Macroscopically, the fiber fraction has a fiber structure principally through the filamentary character of the vascular bundles. Microscopically, in addition to the filamentary structures of vascular bundles and sclerenchyma, typically, pieces of epidermis tissue are also recognizable, consisting of sheets one cell layer thick. The vascular bundles are built up from several cells including wood vessels and sieve tubes. Depending on the extent of fiberization, fibers consisting of one cell occur too, and further the residues of cell walls and (spiral, reticulate or ring-shaped) cell wall thickenings. Typical of the epidermis sheets is the presence of stomata and silicious teeth or hairs.

The fiber stream as contemplated by the invention consists substantially exclusively of a wet solid fiber stream (chiefly cellulose and hemicellulose) basically having no nutritive value, since this fraction is not directly, and microbiologically only to a slight extent, digestible. However, the absence of digestibility makes it possible to use the fiber stream for non-food applications, this in contrast to, for instance, the press cake coming from the above-described traditional pressing methods where the press cake is in fact suitable only for fodder applications and would

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soon rot if it were not prepared into food and was eaten or further preserved.

For example, the invention provides the use of a fiber fraction for the production of energy. The fiber fraction contains principally the 5 carbohydrates cellulose and hemicellulose (composed principally of the elements C, H and O), which are eminently combustible and hence can be converted with a high efficiency to useful energy in, for instance, a combined heating and power station, and which may be expected to entail no or minor emission of harmful substances upon combustion. Processing 10 plant material according to a method as contemplated by the invention, followed by the use of the resultant fiber fraction as fuel, will contribute to the reduction of the CO₂ emission, since what is involved here is a non-fossil fuel. Also, as such, the combustion of the fiber fraction will be cleaner for the environment, since the fiber fraction is hardly, if at all, 15 contaminated with the salt residues (such as K, Na, Cl, P compounds) and protein residues (which include S and N compounds) normally occurring in dry plants. These salt residues and protein residues, coming from the cytosol, have been separated, along with the juice stream, from the fiber fraction. Combustion of the fiber fraction (having therein principally C, H 20 and O compounds which are converted by combustion to H₂O and CO₂) will therefore entail a much lesser environmental impact than combustion of other plant material in which all these salt residues and protein 25 residues are still present. Protein combustion contributes in particular to the emission of sulfur and nitrogen compounds such as sulfur and nitrogen oxides, and incombustible salt residues will contribute to the residual ash volume. Upon combustion of a fiber fraction according to the invention, the emission of, for instance, sulfur and nitrogen oxides, and the residual ash volume having therein the salt residues will be much smaller.

Since the fiber material is of organic origin, it is also applicable, for 30 instance, as a peat substitute in, for instance, potting soil or in horticultural substrates.

In a preferred embodiment of the invention, the plant material is fiberized to such an extent that, for instance, the fiber material consists principally of elemental fibers, so that the so obtained fiber component or fiber stream is suitable, for instance, for further processing into cardboard and/or paper, or can be used as (natural) fiber in composites together with and in reinforcement of (artificial) resins.

Examples of vegetable material that can be treated with a method according to the invention are known (fodder) crops such as grasses (grains such as wheat, rye and maize included), lucerne, hemp, but also 10 harvest residues of crops whose leaf and/or stem parts are normally not processed, such as potato or (sugar) beet tops which are generally left behind in the field upon harvesting; or crops which are generally not processed into juice, or are so processed on a limited scale only, such as spinach, lettuce and grass. The high efficiency of a method according to 15 the invention renders the processing of such vegetable materials profitable.

The juice stream of plant materials processed according to a method contemplated by the invention is further treated, for instance through screening, whereafter, for instance, the protein, peptides, amino acids, and 20 other components or content substances in the juice are recovered by, for instance, coagulation through, for instance, acid and/or heat treatment. The juice stream may also be further processed by (ultra or membrane) filtration, drying, fermentation, or other methods known to those skilled in the art. Protein-rich or otherwise high-grade nutrients for human and 25 animal consumption, but also pigments such as carotene (provitamin A) can be recovered from cytosol in this way, also from that of leaf and/or stem parts.

Also eligible for processing in a method according to the invention is vegetable material not belonging to cultivated crops in the strict sense of 30 the word, such as roadside grass mown along roads or highways, or mixtures of grasses and other wild plants mown in natural areas.

The invention further provides a method for separating components from vegetable material which has been harvested a relatively long time ago and has already, at least partly, dried out, or which can no longer be qualified as fresh and green, but has acquired a more woody and/or dry 5 character for instance through maturation. Such material is not suitable for processing in a pressing method, but is now outstandingly processable, since the extent of turgor of the plant cell to be accessed is not important when a method according to the invention is used.

The invention provides a refiner, or an apparatus of comparable 10 action, and the use of such an apparatus, for instance for separating components from vegetable material which does not (yet) exhibit any lignification, or exhibits only a minor extent of lignification, and in which parenchyma is present. This parenchyma with the cytosol present therein is the basis of the juice stream as contemplated by the invention. A refiner 15 is generally used to break down wood chips into fibers for the purpose of making pulp for the production of paper and/or cardboard. The invention provides for the processing by means of a refiner, of a crop selected from a large variety of crops which traditionally have not been processed with a refiner. Refiners are generally not used for fresh and/or green material, 20 since wood consists principally of dead or lignified tissue from which most parenchyma, with chloroplasts, has disappeared. Different types of refiners are known to those skilled in the art. There are, for instance, refiners with conical disks or with flat disks. The invention provides for 25 the use of both types, and/or equivalent apparatuses, for instance convex/concave type composite grinding disks, in a method provided by the invention.

The invention will now be further explained in the experimental section of the description, without limiting it.

30 Experimental section

Experimentally, the invention was compared with the traditional technique. This was done using a lab(oratory) protocol and industrial equipment. On the basis of that, the nature of the fiber fraction can be assessed and the recoverability of content substances in the two methods 5 can be compared. Results shown hereinbelow illustrate the difference in the recoverability of protein and other content substances.

Traditional method

In the experiments on a laboratory scale, the traditional method of 10 grinding and pressing was simulated by pulping material in a Tecator Homogenizer and squeezing the pulp using an adapted draw pressure bench of Lloyd Instruments. It was provided with a cup having a perforated bottom plate (surface 50 cm²) in which 100 g of fresh pulp were pressed for 15 minutes at a pressure running up to 10 bar. The original 15 material and the press juice were analyzed for nitrogen content, and the recoverability of protein was calculated as the amount of crude protein (amount of nitrogen multiplied by 6.25) in juice expressed as a percentage of the amount of crude protein in the original material.

20 On a larger scale, a hammer mill of the type Jenz A30 was employed to disintegrate grass and the thus obtained grass pulp was squeezed in a Vetter screw press with a compression ratio of 1:7.65 and a perforation of the cylinder wall of 0.7 mm. By passing plant material through the hammer mill a single time or several times, the material could be 25 disintegrated to a greater or lesser extent.

New method

In the experiments on a laboratory scale, the new method was simulated 30 by fine-chopping fresh grass in a cutter, then mixing 30 g of fine-cut grass pieces with 400 ml of water, and fiberizing same in a blender (Braun mixer) for 10 minutes, screening the slurry from the blender on an 850

micron screen, and washing and drying the screened-off fiber fraction. The fiber was analyzed for contents of nitrogen, ash and cell walls and thus the composition of the defibered slurry was calculated. The fiber yield was determined as the amount of dry matter in the fiber fraction as a percentage of the amount of dry matter in the starting material. The recoverability of protein was calculated as the amount of crude protein in the defibered slurry expressed as a percentage of the amount of crude protein in the original material.

10 The new method was also tested with a Sprout-Waldron 12 inch pressure refiner, with grinding disks of the type D2A505. Refining or fiberizing fresh grass was done under atmospheric conditions at a disk distance of 0.04 mm, with addition of water to a consistency of about 2% dry matter. The fiber was then screened on a screen with 140 micron openings.

15 The new method was also tested on a semitechnical scale using a Sunds Disk Refiner type RO 20 FLUFF serial no. 3838, year of manufacture 1985, provided with grinding disks with a high or low resistance to throughput. With this refiner, inter alia the effect of disk type and disk distance on throughput and fiber composition was investigated.

20 Refining occurred under atmospheric conditions with chopped grass, with or without addition of water. The fiberization of potato tops was also tested.

25 The grass originated from both cultivated grassland and natural grounds and was processed in fresh, chopped form. Samples of the fiberized material were rinsed by hand and screened and analyzed for nitrogen and ash content. The recoverability of crude protein was calculated on the basis of an average fiber proportion of 33% of the grass dry matter.

The potato tops originated from starch potato plants during the full growth phase of the potato plant. The tops were pulled mechanically and were consequently crushed to some extent. The potato tops consisted of stems and leaves. The potato tops, without prior washing, were processed 5 with the refiner while fresh, without addition of water. The fiberized material was squeezed by hand.

Experimental results:

Description of the figures

5 Figure 1 and Figure 2 (detail)

Press cake of grass (left) and grass fiber (right) stemming from perennial ryegrass (*Lolium perenne*).

In the press cake, the green color due to the presence of chloroplasts is conspicuous. Also, leaf fragments are recognizable by their size (cross section greater than 1 mm) and the characteristic ribs on the top of the leaf. The grass fiber is distinguished by the light color (virtually complete absence of chloroplasts), the filamentary structure and the small diameter of the individual fibers (in this case very much smaller than 1 mm). The distance between successive numbers is 1 cm.

15

Figure 3

Suspension of grass fiber from perennial ryegrass (*Lolium perenne*).

20 Visible are fibrous structures (vascular bundles) of a diameter of a few tens of micrometers and epidermis sheets of a smallest diameter of up to a few hundreds of micrometers.

Figure 4

25 Microscopic recording of epidermis in grass fiber originating from perennial ryegrass (*Lolium perenne*).

Characteristic is the presence of stomata in perennial ryegrass, concentrated in the epidermis of the top of the leaf. The more compact tissue on the side of the stomata is underlying sclerenchyma. The elongate epidermis cells have a cross section of about 20 micrometers.

Figure 5

Microscopic recording of vascular bundles in grass fiber originating from perennial ryegrass (*Lolium perenne*).

5 Characteristic of vascular bundles are their being built up from several cells and the presence of vessels with reticulate thickenings. The diameter of the fiber in the middle of the figure is about 50 micrometers.

Figure 6

10 Microscopic recording of parenchyma cells in the juice stream of defibered grass originating from perennial ryegrass (*Lolium perenne*). This juice stream belongs to the fiber fraction of Figures 1 and 2.

Characteristic of parenchyma cells in grass leaves is the abundant presence of chloroplasts. Some parenchyma cells, however, have been 15 broken during fractionation: only the cell wall is still visible, the chloroplasts occur in isolation in the surrounding fluid. The size of these parenchymal cells is about 20 * 40 micrometers. The fraction shown in this figure was diluted prior to being photographed to bring out the relatively large amount of parenchyma cells in the juice stream according 20 to the invention.

Figure 7

Microscopic recording of parenchyma cells in press juice from grass originating from perennial ryegrass (*Lolium perenne*).

25 This press juice belongs to the press cake of Figures 1 and 2. The fraction shown in this figure was concentrated prior to being photographed to bring out the relatively small amount of parenchyma cells in the press juice.

30

Figure 8

Process diagram for fiberizing or refining grass.

Figure 9

Process diagram for fiberizing or refining grass.

5 **Figure 10**

Process diagram for fiberizing or refining grass.

Fiberization

Table 1. Fiber composition and fiber yield of cultivated grasses, by species and variety, on average during the season, and of a few other crops.

5

Species/variety	Nitrogen content (g/kg dm**)	Ash content (g/kg dm)	Cel wall content (g/kg dm)	Fiber yield % of dry matter in raw material
Grasses				
<i>Lolium perenne</i> 4n Vr.*	4.0	50.6	867	28
<i>Lolium perenne</i> 2n Vr.	4.3	43.5	865	34
<i>Lolium perenne</i> 4n Lt.	4.5	41.1	879	29
<i>Lolium perenne</i> 2n Lt.	5.4	34.7	857	29
<i>Lolium multiflorum</i> 4n	3.8	47.4	877	24
<i>Lolium multiflorum</i> 2n	4.4	36.6	880	27
<i>Phleum pratense</i>	4.3	39.8	862	30
<i>Festuca arundinacea</i>	4.4	36.7	867	29
<i>Dactylis glomerata</i>	5.1	42.0	873	32
<i>Festuca pratensis</i>	4.5	44.2	872	32
Other plant materials				
Lucerne	5.7	18.9	824	28
Potato tops young	4.2	26.1	836	16
Potato tops old	3.7	50.7	714	21
Pea tops	4.8	25.7	832	29
Beet tops	12.0	79.7	680	9

*) 4n = tetraploid; 2n = diploid;

Vr. = early-flowering; Lt. = late-flowering

10 **) dm: dry matter

5 Fiberizing vegetable biomass yields a fiber fraction which, depending on the nature of the material, can vary from less than 10% to more than 30% of the dry matter. The exact number is also dependent on the mesh of the screen with which the fiber is separated and the intensity of washing. The fiber fraction in the case of *Lolium perenne* typically consists for more than 80% of cell wall material and has a nitrogen content mostly lower than 6-8 g per kg of dry matter and an ash content mostly lower than 50-100 g per kg of dry matter.

10 Table 2. Composition of fiber

		refiner	lab protocol
Ash	(g/kg d.m.)	22.3	26.0
Nitrogen	(g/kg d.m.)	5.3	4.4
15 Cell walls (g/kg d.m.)		808	792

The composition of the fiber fraction is comparable for the experiments with the refiner and the experiments according to the lab protocol.

20 Defibration

Table 3. Composition of grass and of the defibered grass slurry.

		Grass	Defibered slurry	
		refiner	lab protocol	
Ash	(g/kg d.m.)	92.6	138	139
Nitrogen	(g/kg d.m.)	31.0	47.4	48.7
25 Cell walls (g/kg d.m.)		544	375	438

30

In addition to the cell content constituents (such as protein), the defibered slurry also contains a part of the cell walls from the plant material. These are substantially the cell walls from the soft parenchymal tissue which disintegrate upon fiberization and subsequently, in defibration, pass the 5 screen as finely dispersed material. The amount present in the defibered slurry is partly dependent on the diameter of the screen orifices.

Table 4. Recoverability of crude protein from cultivated grasses, by species and variety, on average during the season, and of a few other plant 10 materials, upon grinding+pressing and upon defibration.

Species/variety	Grinding+pressing (%)	Defibration (%)
Grasses		
<i>Lolium perenne</i> 4n Vr.	30	95
<i>Lolium perenne</i> 2n Vr.	23	94
<i>Lolium perenne</i> 4n Lt.	22	95
<i>Lolium perenne</i> 2n Lt	16	94
<i>Lolium multiflorum</i> 4n	41	96
<i>Lolium multiflorum</i> 2n	35	95
<i>Phleum pratense</i>	11	94
<i>Festuca arundinacea</i>	21	94
<i>Dactylis glomerata</i>	31	93
<i>Festuca pratensis</i>	17	94
Other materials		
Lucerne	52	95
Potato tops young	51	98
Potato tops old	42	95
Pea tops	16	95
Beet tops	24	95

Defibration yields a slurry mostly containing more than 70%, and preferably more than 80% or 90%, of all crude protein from the vegetable material. This protein can be recovered from it by centrifugation, which may or may not be preceded by heat coagulation.

5

In the traditional method of fractionation, the recoverability of crude protein is mostly less than 50%.

Table 5. Comparison of protein recoverability from grass upon repeated passage through hammer mill followed by pressing in a screw press, and upon fiberization according to the invention.

10

Protein recoverability
(%)

Hammer mill+screw press

Passages through hammer mill

15

1x	28
2x	30
4x	35
8x	43

Fiberization 93-96

20

according to the invention

Even upon repeated disintegration of grass in a hammer mill followed by pressing in a screw press, the protein recoverability was found to be less than half of the protein recoverability measured upon fiberization of grass.

25

The results of the tests with the Sunds Disk Refiner are summarized in Table 6.

Choice of plate type and disk distance determine the extent of fiberization but determine protein recoverability only to a slight extent. A high throughput was possible in combination with a high protein recoverability (in this case >85%) both with protein-rich cultivated grass and with

5 protein-low natural grass.

Potato tops are well processable with the refiner. In the fiber fraction the content of woody fibers is relatively high because the original potato tops consisted not only of leaf tissue but also of stem tissue. The high ash

10 content in the fibers of the potato tops was caused to an important extent by the high sand content in the tops due to not washing the raw material.

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Table 6. Fiber composition and protein recoverability upon accessing grass and potato tops on a semitechnical scale using a
Sunds Disk Refiner.

Raw material	composition raw material			disk		through-put	composition fiber	protein recoverability
	d.m.	ash	N	Plate resistance	disk distance			
	(g/kg fresh)	(g/kg d.m.)	(g/kg d.m.)	mm	(kg d.m./hour)	(g/kg d.m.)	(g/kg d.m.)	(%)
cultivat. grass	154	91	19.3	high	0.4	-	13	5
cultivat. grass	142	183	36.1	high	0.10	39	31	14
"	"	"	"	high	0.50	55	27	15
"	"	"	"	high	1.00	104	38	15
"	"	"	"	low	0.05	157	49	14
"	"	"	"	low	0.10	135	41	14
"	"	"	"	low	0.50	139	54	15
"	"	"	"	low	1.00	211	82	20
natural grass	215	138	12.1	low	0.10	-	41	6
potato tops	104	342	23.5	high	0.20	-	473	15.2
"	119	344	27.0	low	0.20	-	374	19.0

Process diagrams for refining grass

Pretreatment

5

The appended process diagrams (see Figures 8 to 10) start from the supply of chopped grass as is also conventional in the processing of grass and lucerne in herbage dryers. Normally, the chopping length is in the order of magnitude of a few centimeters, but it can also be longer or shorter. For the 10 refiner test, fresh grass was pre-commminuted in a Pierret guillotine cutter to 6 mm particle length, in other words, very short. Presumably, such a short length is not requisite; refining or fiberizing squeezed grass (of a particle length of presumably a few centimeters) did not present any problems.

15 Washing

A washing step will probably be necessary in practice to remove sand and thereby reduce equipment wear and enable a cleaner product yield. This washing step, however, may be skipped if sand and other contaminants are 20 not present.

Sulfite addition

25 Addition of sulfite may be necessary, but need not be so, to prevent undesirable complexing between proteins and polyphenols. On the basis of past experiences regarding the processing of grass juice, it is known that such complex formation reduces the nutritive values of grass proteins. The circumstances during refining, however, may be different. A rapid temperature rise during refining may instantly stop enzymatic activity 30 (blanching effect) and inhibit formation of polyphenols.

Refining: basic diagram (Fig. 8)

Refining grass is in principle possible with and without liquid addition during refining. In a first test, with fresh grass (15% dry matter), the 5 process did not proceed readily without generous admixture of water to a dry matter percentage of about 2%. The necessity of liquid addition is probably partly dependent on the type of refiner and the nature of the grass (fibrousness). Pressed grass (26% dry matter) could be refined without water addition. If, and if so, how much water is admixed, has consequences for the 10 temperature rise during refining, and therefore for the extent of protein denaturation and hence for the subsequent steps in the process.

The basic diagram includes, after refining, the process steps: screening out the fiber, heat coagulating the refiner liquid followed by separation of the 15 protein cake by means of a decanter and evaporation of the deproteinized liquid. Two extreme variants of this basic diagram are conceivable: one with a minimal addition of liquid during refining and one with ample addition of liquid. The basic diagram is then changed to variant A (Figure 9) and variant B (Figure 10), respectively.

20

Refining: variant A (Figure 9)

Upon minimal addition of return liquid, possibly a substantial temperature rise will occur during refining: in the test with pressed grass to above 70°C. 25 Protein coagulation and pasteurization will then occur already during refining and possibly a separate coagulation step may then be skipped. In that case, the process diagram is simplified to refining - screening - decanting - evaporation: see variant A on basic diagram.

Refining: variant B (Figure 10)

Variant B: In case of ample addition of return liquid, the temperature rise during refining can remain limited: in the test with fresh grass to about 35° C. As a result, presumably, a part of the protein can remain in solution. In 5 that case, after refining, two alternative routes are conceivable. The simplest one is, after screening out the fiber, to heat-coagulate the liquid and decant. In that case, one protein cake is formed and a deproteinized liquid that can be evaporated (see the basic diagram). A more complex route (variant B) comprises, after screening out the fiber, initial decanting 10 whereby a crude protein cake is obtained (crude, i.e. with admixture of finely divided parenchymal cell walls that pass the screen), followed by heat-coagulation and decanting again. In this second decanting step, a purer protein cake is obtained.

15 Screening out the fibers

For the purpose of screening out the fibers, centriscreens can be employed, as known to those skilled in the art for separating potato fiber. In the test, an inclined screen was used, having stretched onto it a wire gauze with 20 openings of 140 * 140 microns. On a lab scale, a screen with a hole diameter of 850 and 250 microns was used. Experiences with it are that most fibers can be separated on a relatively coarse screen. The finer fiber fraction can be added to the total fiber fraction or, via enzymatic deliquescence, to the molasses, concentrate or juice stream.

25

Washing and drying of fiber

The fiber that is separated by screening may be contaminated with 30 dissolved and suspended substance. Accordingly, washing with

deproteinized return liquid is then necessary, followed by moisture removal through pressing/centrifugation and drying.

Drying protein cake

5

The protein-rich cake which is separated through decanting can be dried in the same manner as is known to those skilled in the art for, for instance, potato protein. In case of the presence of a relatively high lipid fraction, addition of an antioxidant product has an improving effect.

10

Evaporation of deproteinized liquid

The deproteinized liquid can be evaporated to form a sugar-rich syrup.

15

Extended procedure

The basic diagram can be expanded to include processes for the purpose of further refining the crude protein cake. One possible addition is enzymatic deliquescence of the parenchymal cell walls in the crude protein cake. The 20 sugars which this yields can, for instance, be added to the molasses, concentrate or juice stream.

REDACTED

CLAIMS

1. A method for separating components from vegetable material which comprises at least leaf and/or stem parts, characterized in that the material is at the least partly fiberized, and subsequently is separated into a fiber fraction and a juice stream, such that the fiber fraction principally comprises relatively firm tissues such as epidermis, sclerenchyma and vascular bundles, and the juice stream principally comprises soft tissues such as parenchyma, and cytosol.
2. A method according to claim 1, wherein the juice stream comprises chloroplasts.
3. A method according to claim 1 or 2, wherein the material is mechanically fiberized.
4. A method according to claim 3, wherein the material is fiberized by means of a refiner.
5. A method according to any one of claims 1-4, wherein the fiber fraction is separated from the juice stream through screening.
6. A method according to any one of claims 1-5, wherein the vegetable material originates from a cultivated crop.
7. A method according to claim 6, wherein the cultivated crop belongs to the family of grasses.
8. A fiber fraction obtained by a method according to any one of claims 1-7.
9. Use of a fiber fraction according to claim 8.
10. Use of a fiber fraction according to claim 9 for the production of energy or for the production of cardboard and/or paper.
11. A juice stream obtained by a method according to any one of claims 1-7.

12. A juice stream according to claim 11, which contains more than 55%, preferably more than 75%, preferably more than 90% of the crude protein of the vegetable material.
13. Use of a juice stream according to claim 11 or 12.
14. Use of a juice stream according to claim 11 or 12 for the production of food.
15. Use of a juice stream according to claim 11 or 12 for recovering or purifying at least a content substance.
16. An apparatus for carrying out a method according to any one of claims 1-7.
17. An apparatus according to claim 16 which comprises at least a refiner.
18. An apparatus in which a method according to any one of claims 1-7 is carried out.

1/7

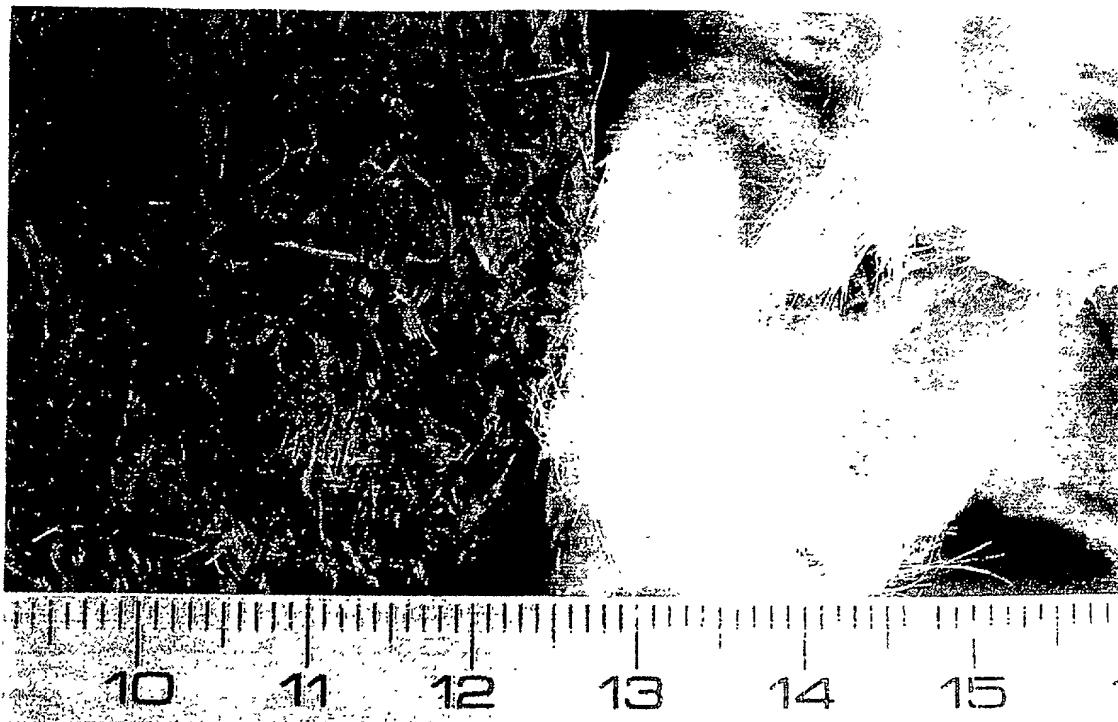


Fig. 1

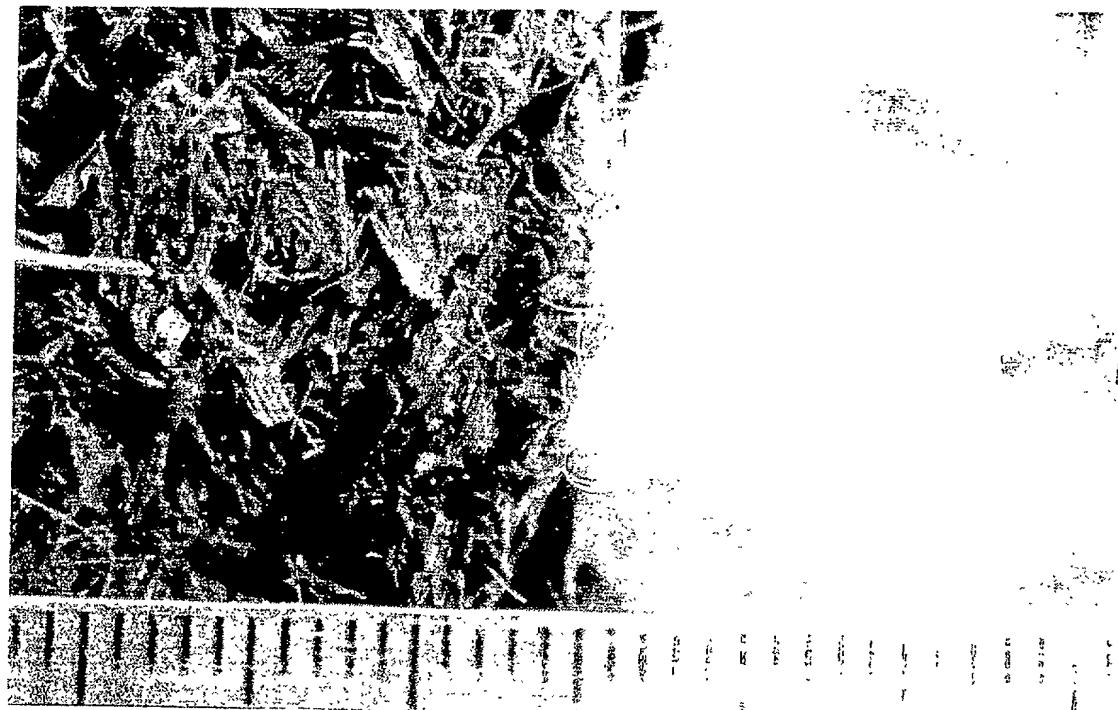


Fig. 2

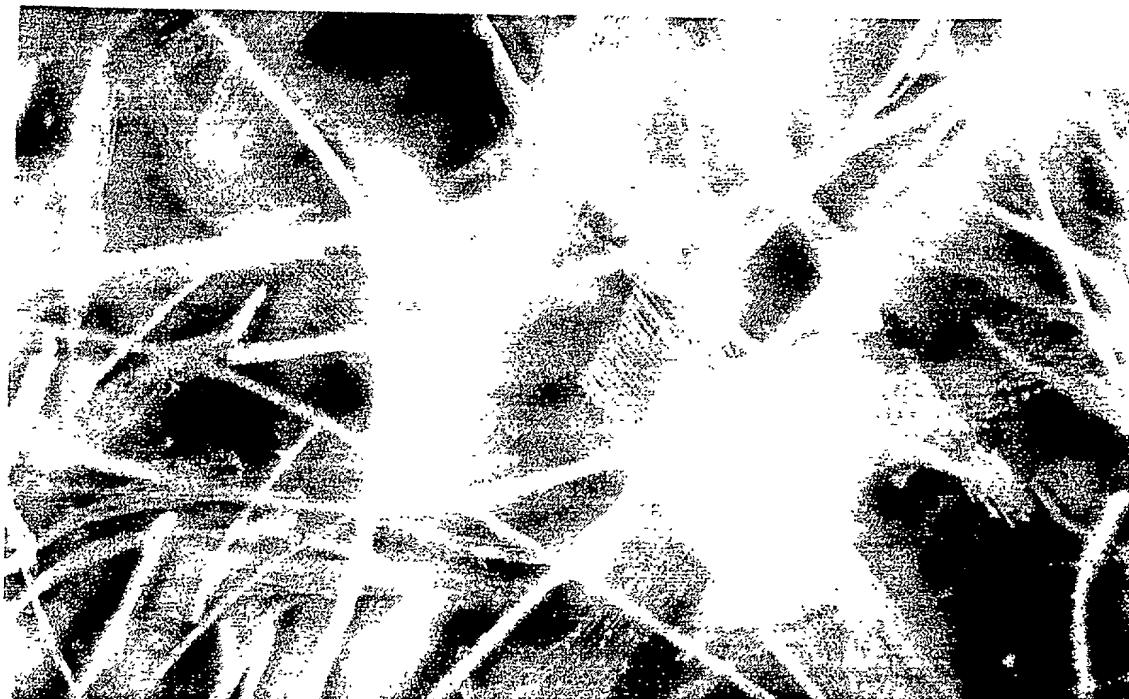


Fig. 3

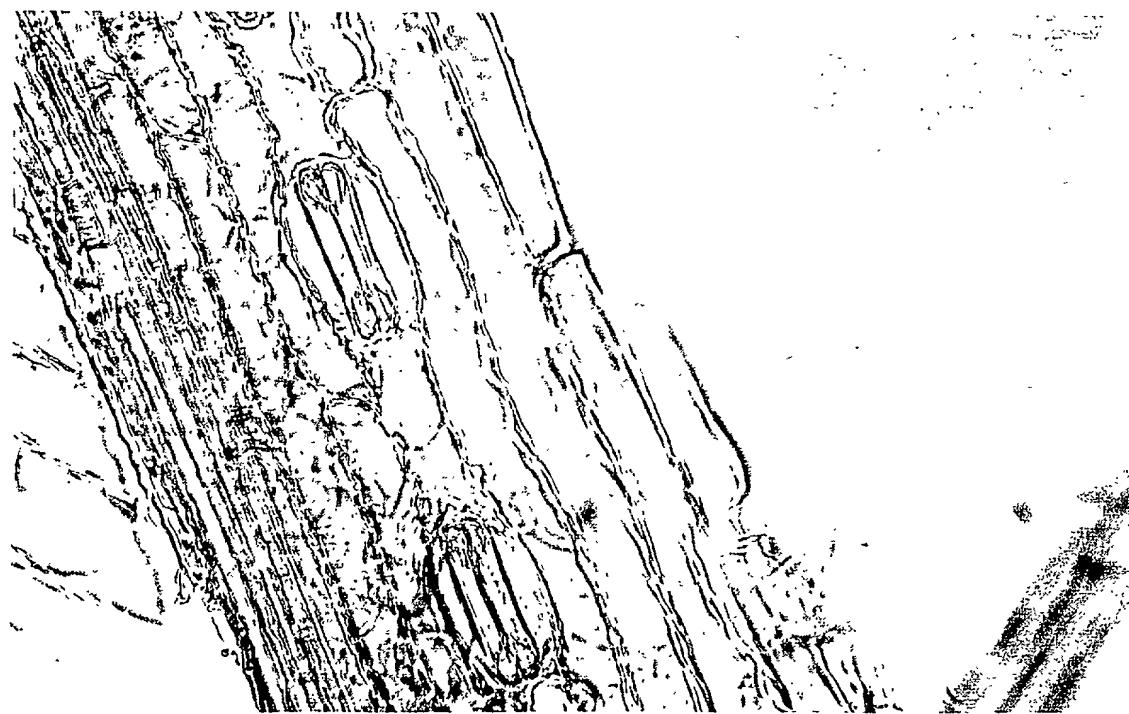


Fig. 4

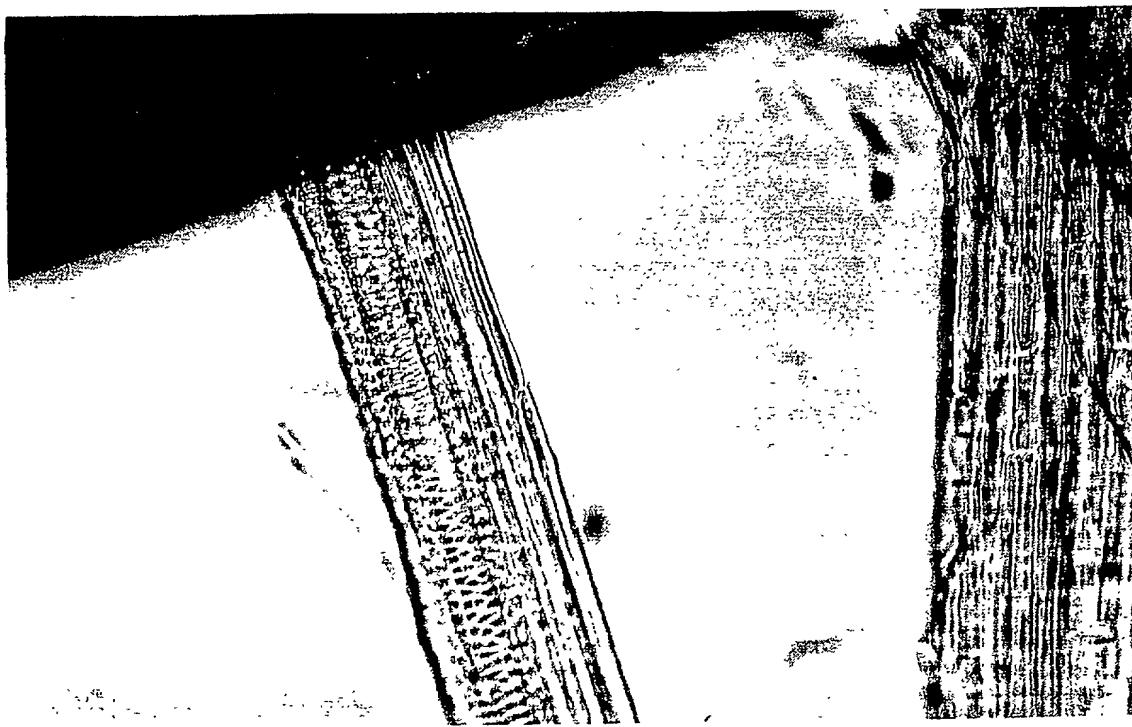


Fig. 5

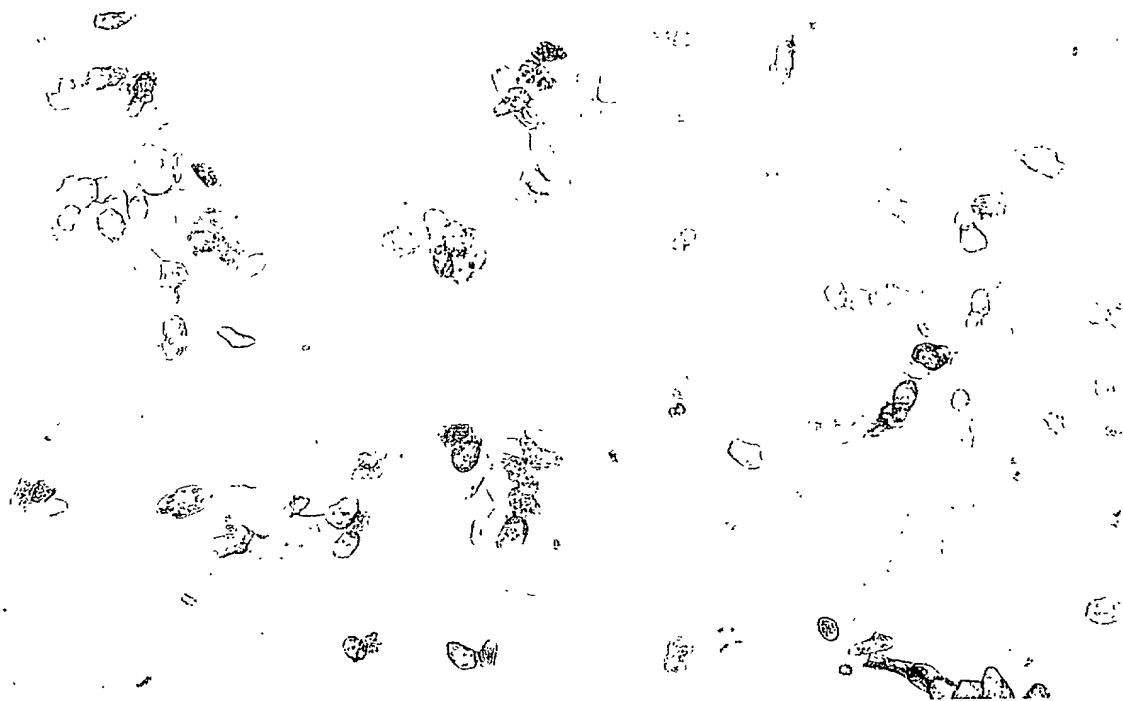


Fig. 6

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1000 900 800 700 600 500 400 300 200 100 0

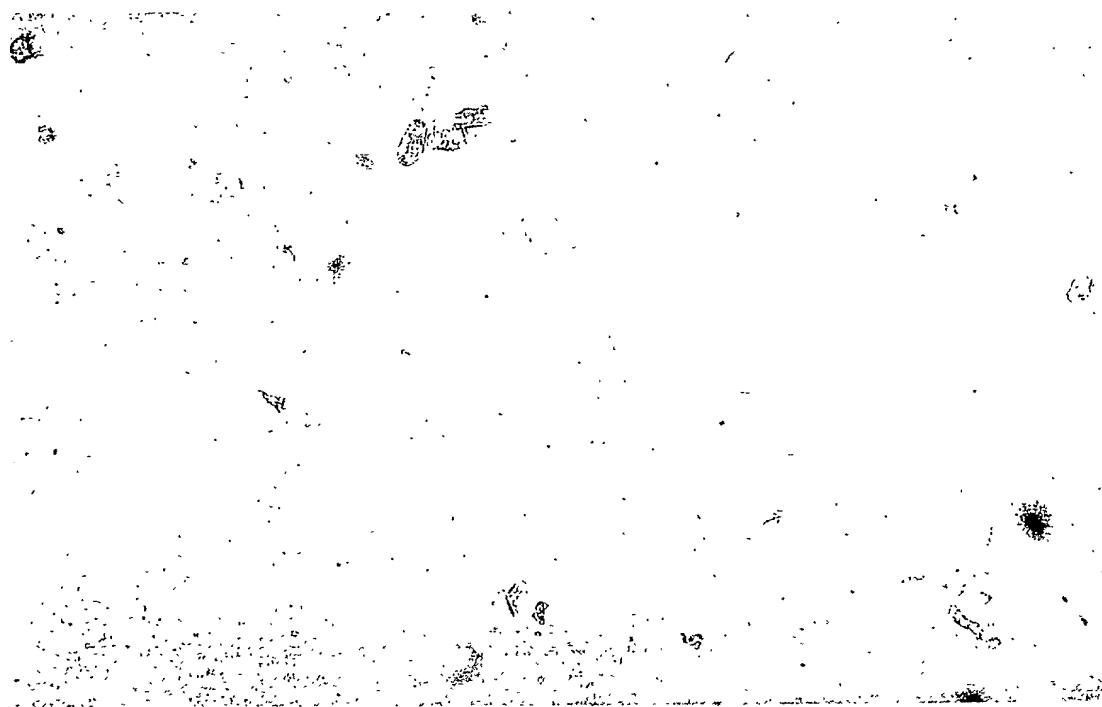


Fig. 7

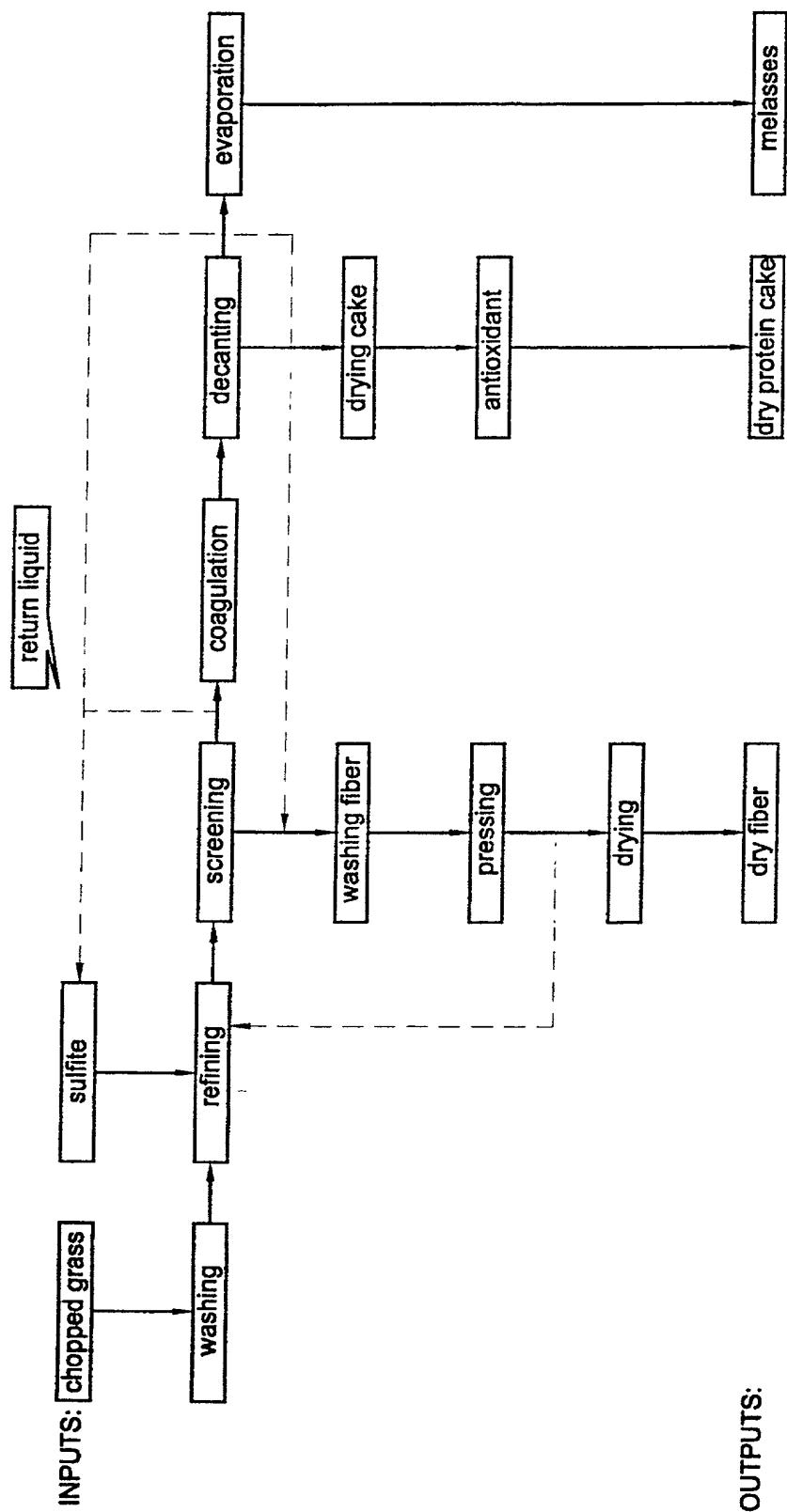
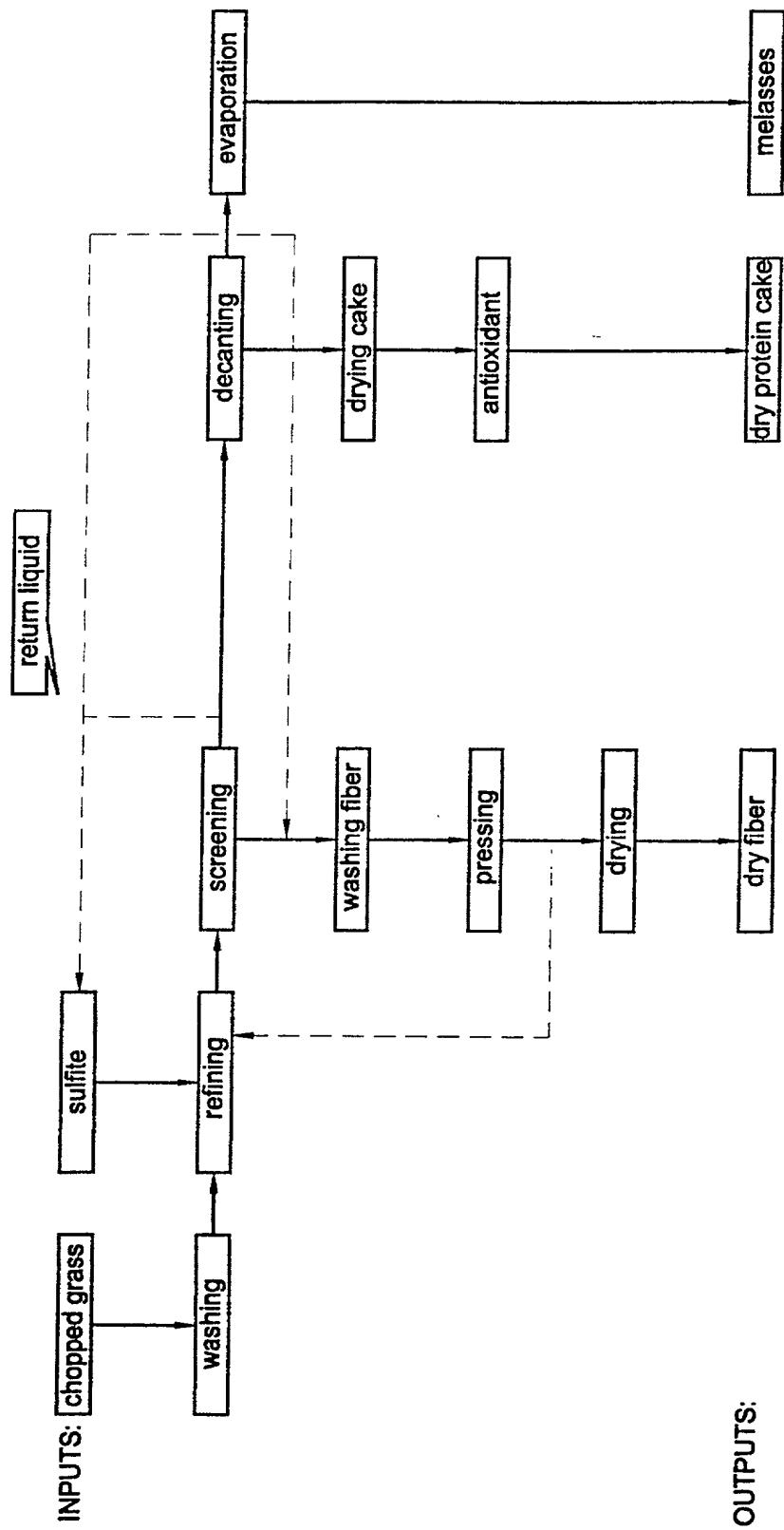


Fig. 8



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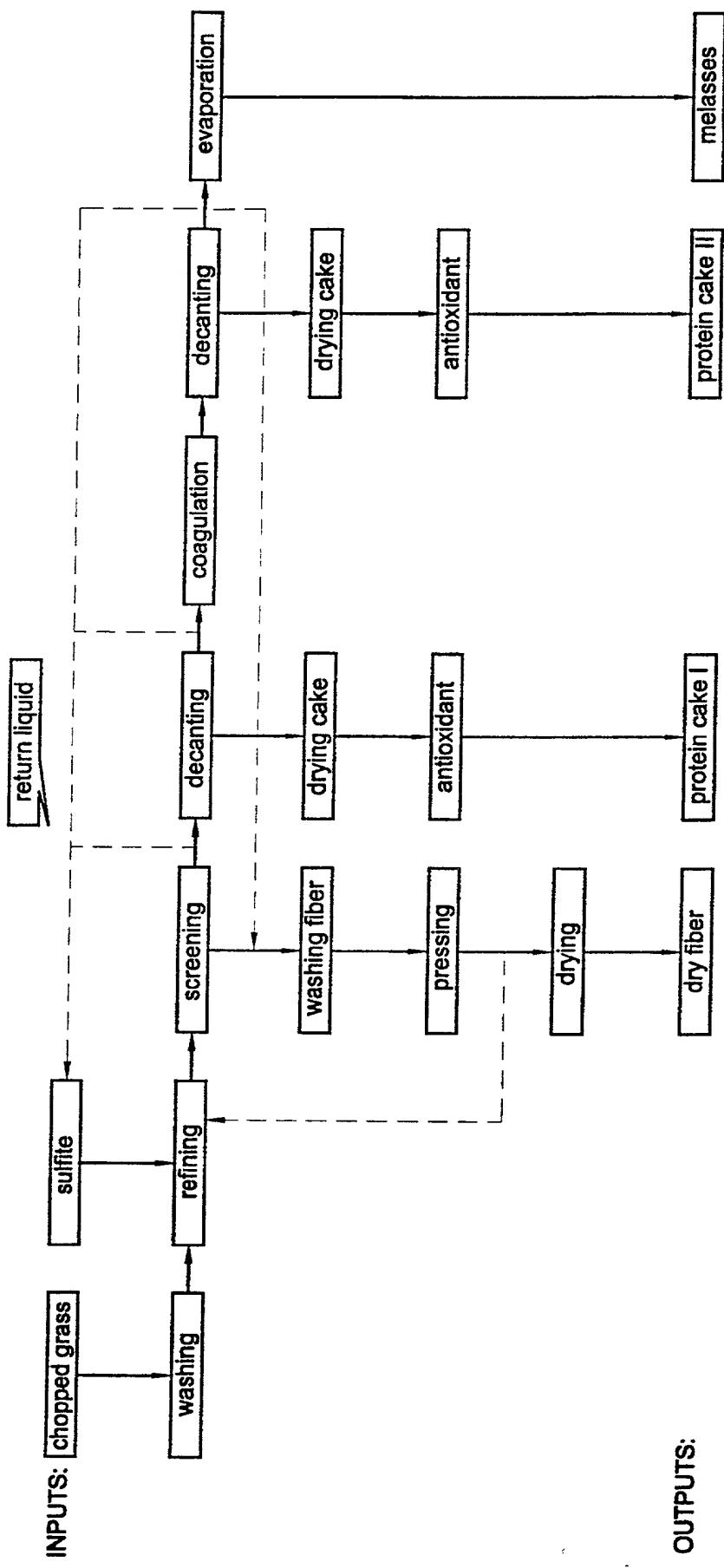


Fig. 10

**Declaration and Power of Attorney Patent Application
(Design or Utility)**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: 'Accessing leaf and/or stem parts of plants'

the specification of which

is attached hereto
 was filed on June 25, 2001 as application serial no. 09/869,409 and or PCT International Application number PCT/NL99/00804 and was amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or 35 U.S.C. §365(b) of any foreign application(s) for patent or inventor's certificate, or 35 U.S.C. §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate of PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)		
Number	Country	Day/Month/Year Filed
1010975	NL	06-01-1999
Number	Country	Day/Month/Year Filed
Number	Country	Day/Month/Year Filed

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

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Serial Number	Day/Month/Year Filing Date
Serial Number	Day/Month/Year Filing Date

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Prior U.S. or International Application(s)		
Serial Number	Day/Month/Year Filed	Status (patented, pending, abandoned)
Serial Number	Day/Month/Year Filed	Status (patented, pending, abandoned)
Serial Number	Day/Month/Year Filed	Status (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Power of Attorney

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

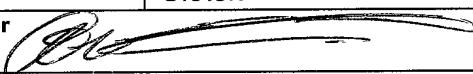
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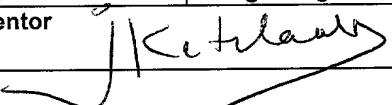
I hereby authorize them or others whom they may appoint to act and rely on instructions from and communicate directly with the person/organization who/which first sends this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instructed otherwise.

Please direct all correspondence in this case to at the address indicated below:

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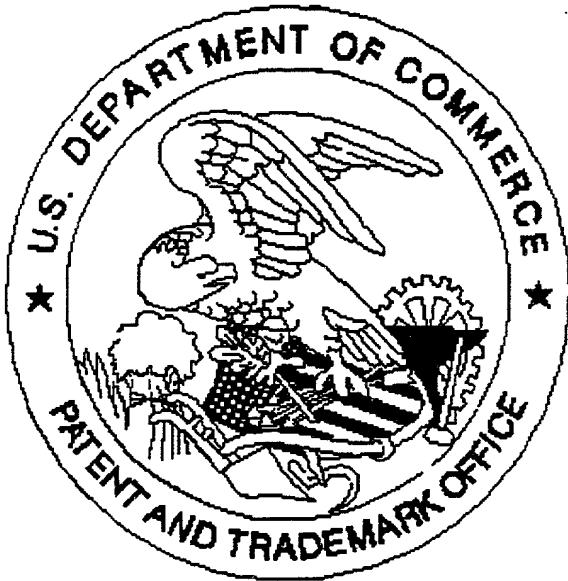
2-00

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